Conference Paper

Genetic Inconsistency in Paternity Investigation

Yudha Nurhantari[1] and Helena Suryadi[2]

[1]Department of Forensic Medicine, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Jalan Farmako, Sekip, Yogyakarta, 55281, Indonesia
[2]Laboratory of Forensic DNA, Eijkman Institute, Jalan Diponegoro 69, Jakarta, 10430, Indonesia

Abstract

DNA fingerprint is one of forensic identification method and has high accuracy. However, genetic inconsistency such as STR mutation in paternity testing may give complexity to the analysis and resolution of the investigation of the case. This study was aimed to analyze the presence of genetic inconsistency or mutation of DNA marker in paternity test cases which came to Department of Forensic Medicine, Dr. Sardjito Hospital/Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada, Indonesia. Totally 58 cases were analyzed from DNA testing cases from 2008 to 2016. Dried bloodstain samples were collected on FTA card after *informed-consent* and DNA extraction was done directly from FTA card. Amplification was done using commercially available kits and genotyping using ABI Prism 3500 for minimum 15 loci of STR, which are D8S1179, D21S11 CSF1PO, D7S820, D13S317, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA. As results, there was a single mutation of STR repeats at FGA and D12S391 loci. The mutation at D12S391 locus is a loss of a single repeat of paternal allele. However, at FGA locus is unknown either loss or gain neither repeats nor occurred in paternal or maternal allele. In conclusion**,** a single repeat mutation was observed at FGA and D12S391 loci.

Keywords: maternal, mutation, paternal allele, paternity test, STR

Corresponding Author:
Yudha Nurhantari
yudhanurhantari@ugm.ac.id
nurhantariyudha@yahoo.com

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1. Introduction

Short tandem repeats (STRs), a simple sequence repeats, known as microsatellite, are short tandemly repeated DNA sequences that involve a repetitive unit 1-6 bp, forming series with lengths of up to 100 nucleotides [1]. Mostly STRs are located in noncoding regions; only about 8 % are located in the coding region [2]. In human, the most common STRs are A-rich units: A, AC, AAAN, AAN, and AG. The STR locus is named as, for example, D12S391, where D represents DNA, 12 means chromosome-12 on which the STR locus located, S stands for STR, and 391 is the unique identifier [1].

Using polymerase chain reaction (PCR), STR loci copied from the human genome, became the genetic markers of choice in forensic DNA analysis for the past twenty

years in large measure because of the multi-allelic nature of STRs produce many possible genotype combinations that can help DNA mixture interpretation [3]. Existing DNA databases, now numbering in the millions of STR profiles, make it less likely that the forensic community will change to different genetic markers in the foreseeable future [4]. Expansion to additional STRs while retaining a connection to legacy STR profile information appears to be the way forward in Europe [5] and the United States [6].

Personal identification is one of the important roles of forensic medicine, including in disputed paternity cases. As the basic principle that the offspring will always receive half of the parent's DNA, it is very clear to determine the biological, parents using DNA fingerprints. However, due to certain reason, sometimes the DNA has a strange result, that might cause different conclusion of the paternity test. As reported in several journals, that some parents show different DNA profile to their offspring. In our institution, the main need of DNA test is disputed paternity. Nowadays, the cost of the DNA test is still high relatively among the people in Yogyakarta and surrounds, so that is the way the number of our clients are still very low. In this study, we reported that two cases of an unusual pattern of DNA inheritance from the parents to their child.

2. Materials and Methods

The samples from 60 DNA testing cases were collected from children and their parents, either trios mother-child-father or duo child-father after informed-consent, which came to Department of Forensic Medicine of Dr. Sardjito Hospital/Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada Yogyakarta, Indonesia from 2008 to 2013. Those blood samples were collected on FTA card and send to DNA Forensic Laboratory, Eijkman Institute, Jakarta. A small punch disc of the FTA card containing the sample can be placed directly into an amplification tube, purified, and amplified, without transferring the disc. A 1.2-mm disc of a bloodstained FTA card was purified using three washes with FTA Purification Reagent and two washes with 1×low-TE buffer. The purified punch disc was then amplified in the MicroAmp® tube for 25 cycles. Amplifications were performed using commercially available STR-kit (AmpFlSTR® Identifiler® PCR or AmpFlSTR® NGM^{TM} Amplification Kit, Applied Biosystems), for minimum 15 STR loci, which are D8S1179, D21S11 CSF1PO, D7S820, D13S317, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA. Amplification program is [7] : initial incubation step temperature was 95 °C for 11 min, followed by 25 cycles of denaturation at 94 °C for 1 min, annealing at 59 °C for 1 min, extension at 72 °C for 1 min, and then the final extend at 60 °C for 60 min, and hold at 4 °C. Samples were analyzed

using the ABI PRISM® 3 500 Genetic Analyzer (Applied Biosystems), according to the manufacturer's recommendations using as separation medium Performance Optimized Polymer (POP) 4TM (PE Biosystems). The data was acquired by v1.0 software ABI PRISM® 3 500 Collection and analyzed by GeneScan® Analysis software 3.1 and Genotyper® 2.5 according to the manufacturer's recommendations

3. Results

A study from 58 cases of DNA testing, 30 cases were matched and unmatched in 28 cases (see Table 1). From matched cases, the inheritance pattern was consistent; half of the loci of each allele were transferred from the parents to the child, except in two cases. The research investigation was showed genetic inconsistency in two cases (see Table 2). From STRs loci analyzed, two loci, those are FGA and D12S391 loci, had a mutation (see Table 3). In a mutation case of FGA locus, the mother's profile is (22, 25), alleged father (22, 23) and child (22, 24). It has 23 repeats of paternal and 25 of maternal. However, it shows up 24 repeats of the offspring. Since 22 allele was present on both the paternal and maternal allele, the mutation may be a single repeat unit gain of paternal or a single repeat unit loss of material.

TABLE 1: Number of matched and unmatched cases.

Matched	38	65.5 %
Unmatched	20	34.5 %
Total	58	100 %

TABLE 2: Number of matched loci in matched cases.

Number of matched loci	Number of tested loci	Number of cases
20	20	12
19	20	1
20	20	4
15	15	9
20	20	4
20	20	3
19	19	1
16	16	1
15	16	1
Total		38

Table 3: Number of mutation in each locus.

Name of loci	Number of mutations
D8S1179	0
D21S11	0
CSF1PO	0
D7S820	0
D13S317	0
THO1	0
D13S317	0
D16S539	0
D2S1338	0
D19S433	0
vWA	0
TPOX	0
D18S51	0
D5S818	0
FGA	1
D12S8391	1
Total number of mutations	2

Table 4: STRs result showing a parent-child mismatch.

Locus	Genotype			Mutation	Gain/Lose
	Father	Child	Mother		
FGA	(22, 23)	(22, 24)	(22, 25)	Unknown	Unknown
D12S391	(19, 20)	(18, 18)	(18, 20)	Paternal	loss

4. Discussion

Mutation can and do occur at STR loci, as with any region of DNA, and STR alleles can change over time [8]. For certain STR loci, all of the alleles that exist today, theoretically have resulted from a few 'founder' individuals by slowly changing over thousands of years [9]. The mutational event may be in the form of a single base change or the length of the entire repeat. The molecular mechanisms by which STRs mutate are thought to involve replication slippage or defective DNA replication repair [8, 10]. The strand-slippage replication appears to be widely regarded as the main pattern of STR mutation compare to other proposed mechanism, which is unequal crossing over in meiosis and retrotransposition mechanism [1].

Estimation of mutational events at a DNA marker may be achieved by comparing genotypes of the parents to the child. An investigation of an allele difference between the parents and the child is seen as evidence for possible mutation. The search for a

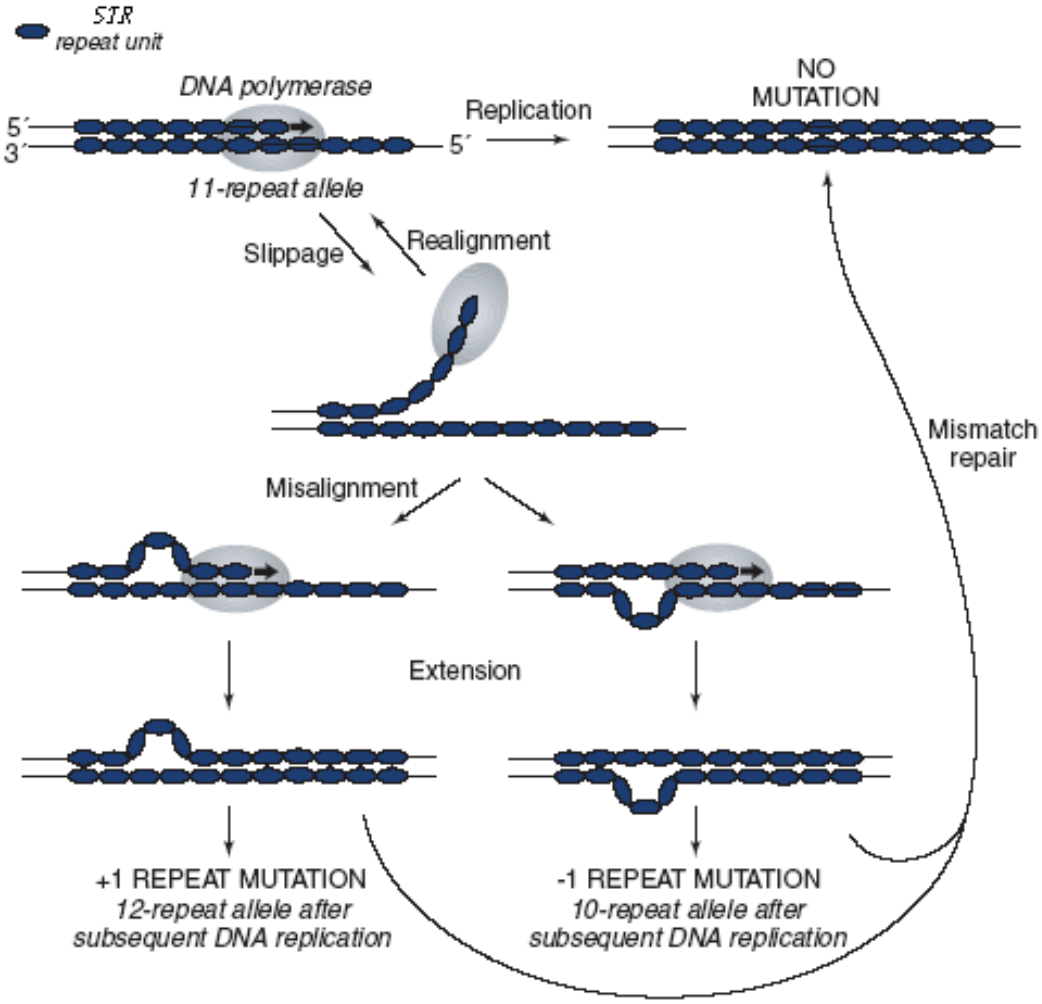

Figure 1: Schematic illustration of the strand-slippage replication at STR [11].

mutation in STR loci involves many parent-child allele transfers because the mutation rate is rather low in most STR [5].

The interpretation of the paternity test may affect by mutation occurred during meiosis, which may result in discrepancy at a locus. In this case, at FGA locus, the mother has a [22, 25] type, the biological father has the type of [22, 23]. The offspring has [22, 24] type. By comparing data at this one locus, it appears that the child is assumed father or mother can be excluded because the [24] alleles were not passed from the parents. The [22] alleles may pass from the mother or father. During meiosis, it is possible for an additional or losses of a unit repeat to the DNA strand. Therefore, the mother or father could have passed along a 24 allele, even though the parents do not have the type of allele if this mutation took place. The FGA locus is a compound tetranucleotide repeat found in the third intron of the human alpha fibrinogen locus on the long arm of chromosome 4 (GRCh38, chromosome 4, location 154587736 to 154587823, reverse strand, allele

designation 22). FGA has also been referred to in the literature as FIBRA or HUMFIBRA. The locus contains a CTTT core repeat flanked on either side by degenerate repeats. The most common motif appears to consist of $[TTTC]_3$ $[TTTT,TTCT,CTTT]_n$ $[CTCC,TTCC]_2$ [12].

D12S391 locus is a highly polymorphic compound tetranucleotide found on the short arm of chromosome 12 (GRCh38, Chromosome 12, location 12297020 to 12297095, forward strand, allele designation 9). It has a repeat pattern as $[AGAT]_{11}$ $[AGAC]_7$ [AGAT,12]. In this case, the father has a profile (19, 20), the child (18, 18) and mother (18, 20). The 18 alleles were transferred from the mother and indicated that mutation occurred on the father, where his 19 alleles had loss a single repeat, so the child got 18 alleles.

Several factors may influence the mutation rate such are number, unit, and structure of the repeat, base composition of the unit, recombination and flanking sequence. One of the key effective factors influencing STR mutation is repeat number. Studies using different methods, such as familial approach [13] and population approach [14], have strongly suggested that STR mutation rate increases with repeat number. Some studies displayed a positive association between mutation rate and repeat number in many vertebrate species [15] including humans [16]. The direction of mutation may be different for alleles of different sizes within a locus [17]. An expansion occurs more frequently in short STRs, while a reduction of repeat number exists in longer ones [18]. The mutation rate of dinucleotide repeats is higher than that of trinucleotide repeats [19, 20]. These results agree with the slippage studies mentioned above. In autosomes, Y chromosome, and tumor cells, it was found that the mutation frequency was appreciably higher in heterozygotes with large allele span [21], indicating that the repeat structure could have some contributions to the STR mutation process. Sequences of the unit with a high AT content mutate faster than those with a high GC content [19], suggesting that the template stability could influence the mutation rate. Perhaps the sequences with high GC content could reduce the frequency of strand-slippage events.

In this study, an only a very small number of cases was studied. It needs more and more sample to be analyzed. However, according to the published data of apparent mutation observed at STR loci, which is adapted from the American Association of Blood Banks, the mutation rate at FGA locus is relatively higher than the other loci [23]. So we can conclude that this case is a mutation, and therefore the alleged father cannot be excluded as the biological father. It is important to develop a procedure to effectively deal with them in paternity cases since the mutation rate is known. Many researchers suggest using two loci exclusion for STR loci because two mutations would not occur at two different loci during meiosis.

Low mutation rates are especially important for paternity testing since the relationship between the child and alleged father are being made based on the assumption that the alleles remain the same when they are passed from tone generation to the next. The high rate of STR marker mutation could result in false exclusion at that locus [8]. However, high mutations rates help keep the STR markers polymorphic and therefore useful in human identity testing. Mutations can potentially impact kinship reference sample; however, they will not affect direct matches between personal effects and victims or perpetrators and crime scene evidence since any mutation that occurs will be consistent over an individual lifetime [7].

5. Conclusion

The result of this study is to investigate two cases out of 58 of DNA STR markers mutation, at FGA and D12S391 loci. It is difficult to determine which of the parents have a mutation in FGA locus since they share the same 22 alleles, and the other alleles have only one unit repeat different. However, in D12S391 locus, single deletion of repeat occurred on the father.

For a suggestion, it needs more sample to be analyzed to investigate other loci mutation determine mutation rate of each locus in our population.

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